EXHIBIT 4

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Tsay et al.

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[54] HEAT TREATMENT OF IGM-CONTAINING IMMUNOGLOBULINS TO ELIMINATE NON-SPECIFIC COMPLEMENT ACTIVATION

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[21] Appl. No.: 504,161

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[52] U.S. Cl. 530/390.5; 424/85.8

[58] Field of Search 424/89.8; 530/387, 389, 530/427, 387.1, 389.1, 390.5; 914/12; 514/2

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S. Barandun et al, Vox. Sang. 7, 157–174 (1962).

M. Wickerhauser et al, Vox. Sang. 119–125 (1972).

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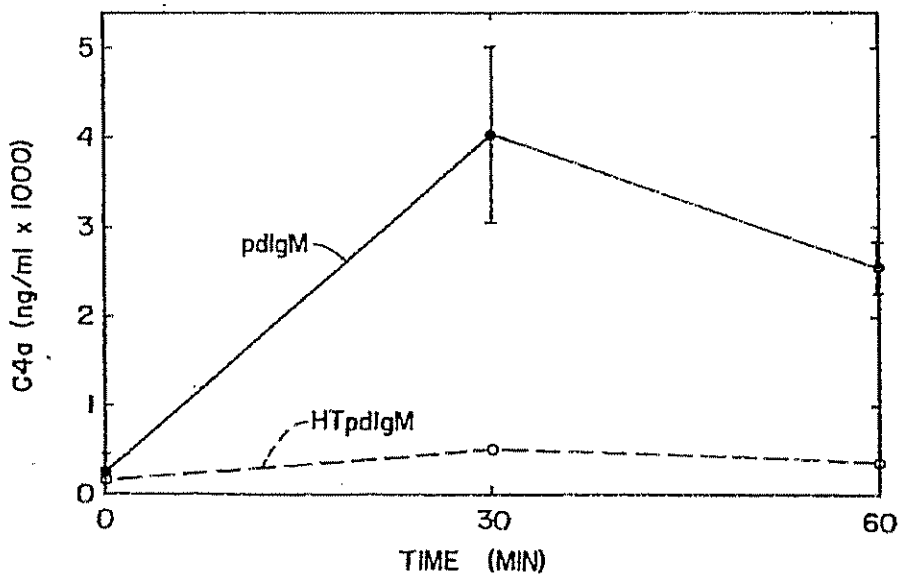
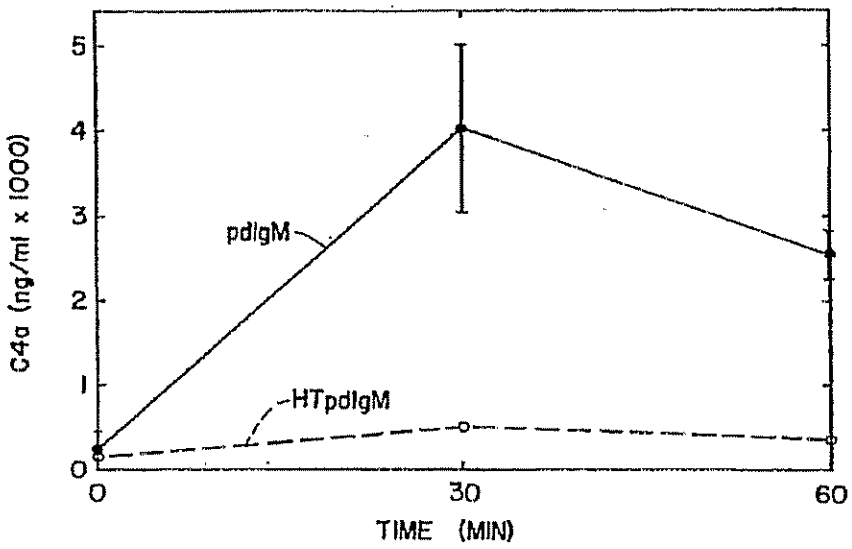
[57] ABSTRACT

Mild heat-treatment of IgM antibody concentrates diminishes the potential to induce non-specific complement activation without significant loss of normal immunologic effector functions. These IgM immunoglobulin concentrates retain specific antigen binding properties and activate complement specific antigen binding properties and activate complement when bound to antigen. Preferred product includes at least 20% by weight IgM in an IgM/IgG antibody mixture. Heating is done at a temperature within the range of about 40° C. to 62 ° C., preferably about 45° to 55° C., in a solution having an acid pH (preferably 4.0 to 5.0) for at least about 10 minutes.

3 Claims, 3 Drawing Sheets

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4
3
2
1
0
pdIgM
HTpdIgM
0
30
60
TIME (MIN)

FIG._1.

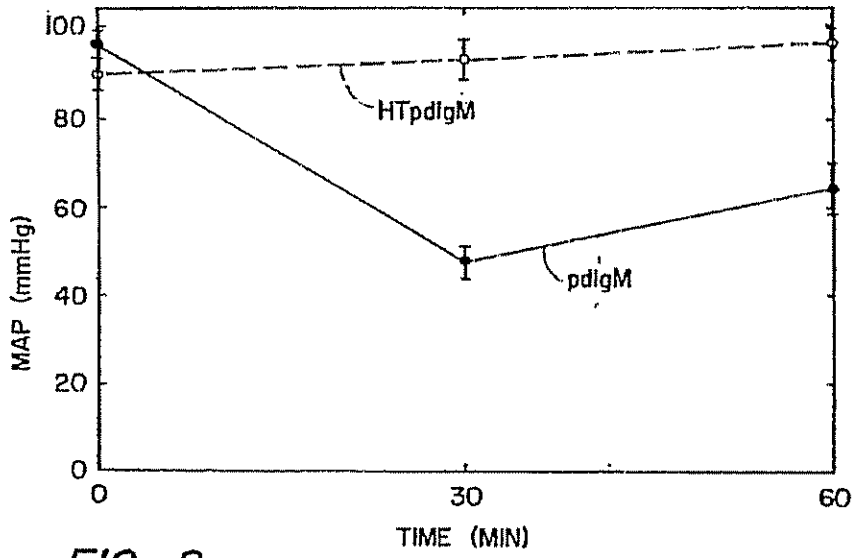
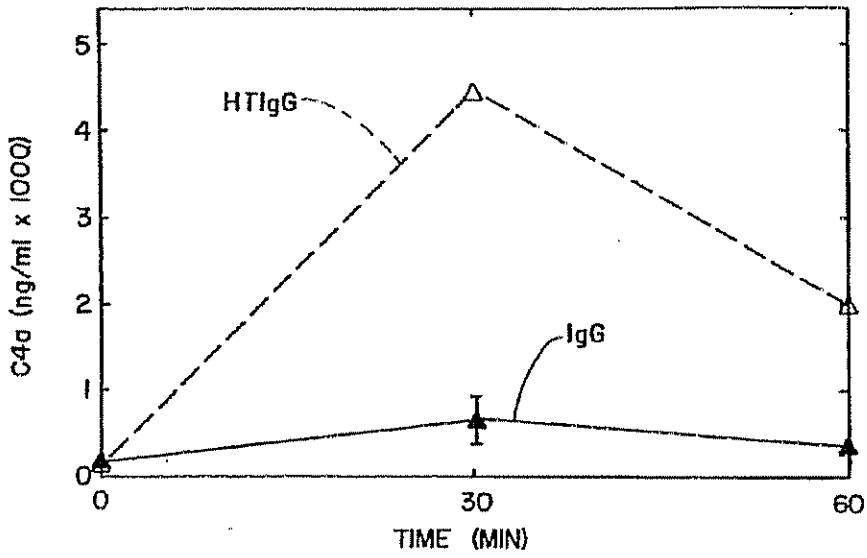

MAP (mmHg)
100
80
60
40
20
0
HTpdIgM
pdIgM
0
30
60
TIME (MIN)

FIG._2.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5
4
3
2
1
0
HTIgG
IgG
0
30
60
TIME (MIN)

FIG._3.

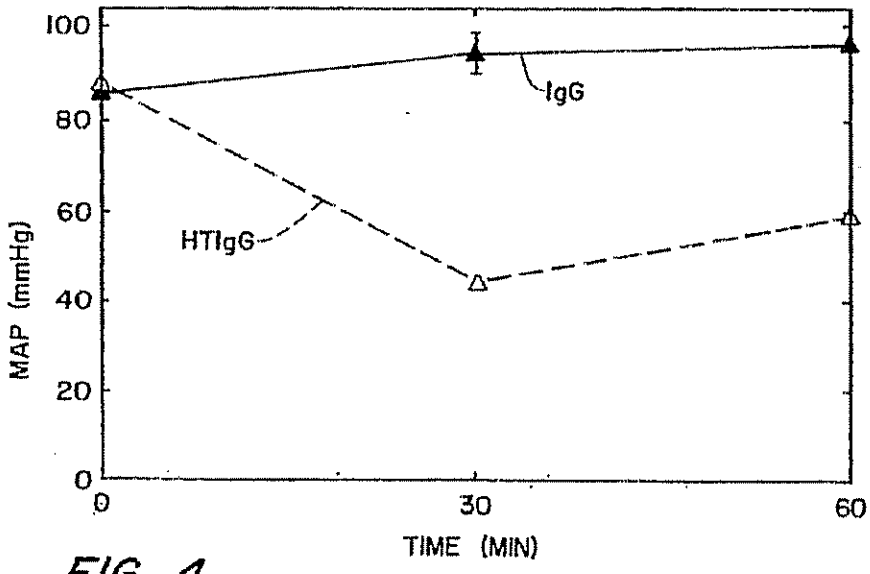

MAP (mmHg)
100
80
60
40
20
0
IgG
HTIgG
0
30
60
TIME (MIN)

FIG._4.

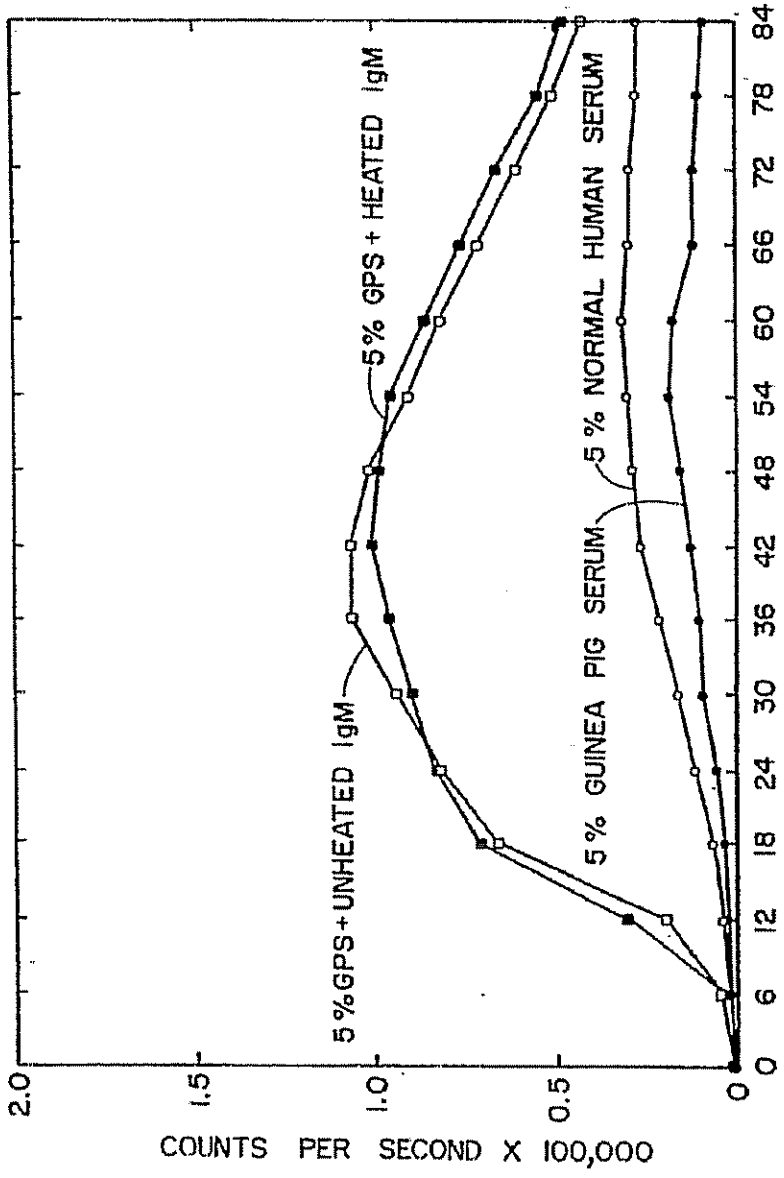

5% GPS + HEATED IgM
5% NORMAL HUMAN SERUM
5% GUINEA PIG SERUM
5% GPS + UNHEATED IgM
TIME (MIN)
0
6
12
18
24
30
36
42
48
54
60
66
72
78
84
COUNTS PER SECOND X 100,000
0
0.5
1.0
1.5
2.0

FIG. 5.

HEAT TREATMENT OF IGM-CONTAINING IMMUNOGLOBULINS TO ELIMINATE NON-SPECIFIC COMPLEMENT ACTIVATION

BACKGROUND OF THE INVENTION

1. Field

This disclosure is generally concerned with therapeutic antibody or immunoglobulin preparations and specifically with therapeutic immunoglobulin preparations that include at least some antibodies of the IgM type.

2. Prior Art

Antibodies may be classified according to a well known typing system (i.e. IgM, IgG, IgA, IgD, IgE) and, in case of IgG, according to sub-types (i.e. IgG_1, IgG_2, IgG_3, and IgG_4).

Commercially available immunoglobulin preparations (known as immune serum globulin or ISG) commonly consist mainly of antibodies of the IgG type with the distribution of IgG sub-types approximating that found in human plasma. Typically, the amount of IgM in such preparations, if present at all, is relatively small.

IgM is a well known 19S immunoglobulin which comprises about 7% of the immunoglobulins found in man. IgM antibodies are said to have an antibody valence of at least five and they are the earliest antibodies generated in an immune response. Although IgM antibodies tend to be very effective, especially in combating bacterial infections, they have a relatively short in vivo half life of about five days. Further, IgM antibodies tend to aggregate and are relatively difficult to stabilize, especially in purified form.

To date, the only known commercial intravenous (IV) product having significant amounts of IgM antibody is a product known as Pentaglobin™, available from Biotest, GmbH, of West Germany. The use of that product appears to be described in articles by K.D. Tympner, et al, "Intravenous IgM-Application," Mschr. Kinderheilk. 123,400–401 (1975) and by K.N. Haque, et al "IgM-Enriched Intravenous Immunoglobulin Therapy in Neonatal Sepsis" Am. J. Dis. Child. 142, 1293–1296 (1988). That product comprises, on a percent by weight total protein basis, about 76% IgG, about 12% IgA and about 12% IgM.

It has been thought that the use of larger amounts of IgM in an ISG product could lead to adverse reactions. For example, it is known that IgM is many times more potent than IgG in activating the complement cascade in an immune reaction. This is because only one molecule of IgM bound to an antigen will activate complement whereas two or more molecules of IgG must be bound to an antigen in close association to each other to activate complement.

It appears that the very production methods used in preparing IgM-enriched products may limit the amount of IgM available due to degradation reactions. See, for example, U.S. Pat. No. 4,318,902 to W. Stephen, describing the use of β-propriolactone to make an IgM enriched product IV administrable. Hence, for whatever reason, even though IgM is recognized as very effective, it has not appeared in any commercially available intravenously useful ISG product at an amount greater than about 12% by weight total protein. Although a 20% by weight IgM product has been available, in the past (Gamma-M-Konzentrat, Behringwerke AG, Marburg, Germany), it has been made for and limited to intramuscular (not IV) applications.

Various purification schemes have been suggested for plasma-derived IgM and, more recently, monoclonal-derived IgM. In the case of plasma-derived IgM, it has been known since the 1940's that alcohol fractionation techniques could be used to obtain a relatively concentrated IgM from what is known as Cohn Fraction III. See also, for example, the above-cited U.S. Pat. No. 4,318,902 (and the cited references) to W. Stephen concerned with the use of beta-propriolactone to make a concentrated (12%) IgM suitable for intravenous (IV) administration. In addition, see EPO application 0 038 667 of Miura et al (IgM acylation). Other IgM purification or preparation techniques are disclosed by U. Sugg et al, Vox Sang. 36:25–28 (1979); M. Steinbach et al, Preparative Biochemistry 3 (4), 363–373 (1973) and A. Wichman et al, Biochem. Biophys. Acta 490:363–69 (1977). For a variety of technical reasons, plasma derived IgM has been relatively difficult to purify and the highest known purity to date (used in analytical purposes) is about 90% IgM, by weight.

In addition to the above problem associated with IgM-rich preparations, it has been observed that the preparations in use tend to generate what is known as non-specific complement activation. Non-specific complement activation refers to the initiation of the complement cascade even in the absence of antibody-antigen complexing. This phenomenon is often associated with the infusion of aggregates of immunoglobulins. Non-specific complement activation is to be avoided since it may cause undesirable side effects such as hypotension. Specific complement activation, on the other hand, is desirable and it occurs only after the immunoglobulin(s) has bound to, for example, the antigenic surface of a microorganism in the bloodstream.

It has been reported by S. Barandun et al "Intravenous Administration of Human Gamma-Globulin," Vox Sang 7, 157–174 (1962) that human gamma-globulin for intravenous administration heated at 37° C. at pH 3.8–4.0 for 24 hours, followed by pH adjustment to 7.0, resulted in a reduction of anticomplementary activity (AC) measured by complement fixation test. However, this treatment for longer periods of incubation resulted in high anticomplementary activity due to the formation of aggregated gamma-globulin. These authors did not demonstrate retention of specific complement activity by the heated immunoglobulin when bound to antigen. Furthermore, no demonstration of in vivo safety was reported by these authors. In addition, M. Wickerhauser et al "Large Scale Preparation of Macroglobulin," Vox Sang 23, 119–125 (1972) demonstrated that IgM concentrates prepared by PEG precipitation had high anticomplementary activity (AC) by standard complement fixation test and this AC ativity was reduced 10 fold by incubating the IgM concentrate at pH 4.0 at 37° C. for 8 hours followed by readjustment to neutral pH. Similar to the previous paper (Vox sang 7, 157–174 (1962), these authors did not assess the specific complement activating potential of the heated IgM concentrate, nor did they assess safety in any animal model.

We have now found that the problem of non-specific complement activation associated with IgM or IgM rich immunoglobulin preparations can be minimized (without losing specific complement activation) in a relatively simple and surprising way.

SUMMARY OF THE INVENTION

Our method of substantially eliminating non-specific complement activation in an IgM-containing immunoglobulin preparation while retaining specific complement activation effector functions comprises the step of subjecting the preparation to a gentle heating step under conditions sufficient to eliminate the non-specific complement activation while not adversely affecting the normal biological activity or antigen binding ability of the IgM antibody. To do this, we have found that the heating step should be at a temperature ranging from about 40° to 62° C., preferably about 45° to 55° C. for at least about 10 min. and the preparation should be in an aqueous solution having an acid pH ranging preferably from about 4.0 to 5.0. To date, the preferred temperature appears to be at or very close to 50° C. for at least about 30 minutes.

Our improved product comprises an immunoglobulin preparation which includes at least some measurable antibodies of the IgM type. On a dry weight basis, a preferred product comprises at least 20% antibodies of the IgM type, the remainder of the antibodies being mainly of the IgG type. Trace amounts (less than 20% by weight) of other types may be present. Details of our preferred product and processes are given below

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 represents plasma $C4_a$ anaphylatoxin levels in monkeys infused with plasma derived IgM (pdIgM) or heat-treated plasma derived IgM (HT pdIgM).

FIG. 2 represents mean arterial blood pressure (MAP) measurements in monkeys infused with plasma derived IgM (pdIgM).

FIG. 3 represents plasma $C4_a$ anaphylatoxin levels in monkeys infused with native intravenous gamma globulin (IgG) or heat-treated intravenous gamma globulin at neutral pH (HTIgG).

FIG. 4 represents mean arterial blood pressure (MAP) measurements in monkeys infused with native intravenous gamma globulin (IgG) or heat-treated intravenous gamma globulin at neutral pH (HTIgG).

FIG. 5 represents ability of unheated or heated IgM to promote phagocyte chemiluminescence against *E. Coli* 0.50:kL bacteria.

SPECIFIC EMBODIMENTS

Work in our laboratory has demonstrated a reproducible adverse response elicited by infusion of IgM-enriched, IgG immunoglobulin concentrates in the pentobarbital-mesthetized cynomolgus monkey. That product consisted of about 50% by weight IgM on a dry weight basis, the remaining being IgG. The mixture was given IV as a 5% aqueous solution. The rate of administration was 1 mg/kg/min (IgM) to a total dose of 50 mg/kg. A major component of the adverse response was a severe decline in arterial blood pressure. In attempting to understand the mechanism of the adverse effect, we demonstrated that heat-aggregated IgG prepared at neutral pH (not acid pH) when infused in the monkey elicited effects remarkably similar to those observed following the infusion of IgM-enriched, IgG immunoglobulin concentrates. Since both the IgM-enriched, IgG immunoglobulin concentrates and aggregates of IgG formed at neutral pH are capable of activating the classical pathway of complement, we hypothesized that complement activation is associated with elicitation of the adverse effect in the cynomolgus monkey. The classical complement pathway is described in Inflammation: Basic Principles and Clinical Correlates Complement: Chemistry and Pathways, pp 21–53, the teachings of which are incorporated herein by reference (Raven Press, NY, N.Y., 1988).

The complement system functions primarily as an effector mechanism in the immune defense against microbial infection. The activated products of the complement system, attract phagocytic cells and greatly facilitate the uptake and destruction of foreign particles by opsonization. There are two pathways for activating complement, the classical pathway and the alternate pathway. Activation of the classical pathway is initiated by antigen-antibody complexes or by antibody bound to cellular or particulate antigens. The alternate pathway is activated independent of antibody by substance such as bacterial wall constituents, bacterial lipopolysaccharides (LPS), cell wall constituents of yeast (zymosan) and Fungi. It is thought that the alternate pathway provides protection against infection prior to an immune response whereas the classical pathway is important after antibody production has occurred.

Activation of the blood complement system generates bioactive peptide fragments called anaphylatoxins. Complement 4a (C4a) anaphylatoxin is a split product of C4 (MW 8740). When C1q is activated by antigen-antibody complexes or aggregates, the C1 complex splits C4 into C4a and C4b allowing C4b to bind to the activating surface while C4a anaphylatoxin is released into plasma. Recent developments in analytical biochemistry have provided techniques which permit the measurement of plasma C4a by radioimmunoassay. See, for example, U.S. Pat. No. 4,731,336 and European Patent 97,440 both to P.S. Satoh

Determination of $C4_a$ levels in plasma provides direct information regarding activation of the classical complement cascade in vivo. Furthermore, the induction of C4a generation in vitro, by various immunoglobulin preparations using human serum as the complement source, is correlated with in vivo complement activation in the monkey following infusion of the immunoglobulins

In the studies described herein, we determined whether adverse effects (hypotension) elicited by IgM-enriched, IgG immunoglobulin concentrates and/or heat-aggregated IgG formed at neutral pH are associated with elevated levels of plasma C4a. In addition, non-specific activation of complement (classical pathway) induced by the immunoglobulin preparations was assessed by C4a generation in vitro.

Using these assay systems, we, furthermore, demonstrate that mild heat-treatment of IgM enriched, IgG immunoglobulin concentrates diminishes C4a generation in vitro and correspondingly this mild heat-treatment diminishes adverse side effects (hypotension) associated with parenteral (IV) administration in the non-human primate. Finally, we demonstrate that the mild heat-treatment process step did not significantly effect the antigenic determinants of either IgM or IgG or the specific antigen binding sites; thus the effector functions of the immunoglobulin are unaltered. Retention of the desired specific complement activation properties of the immunoglobulin was confirmed in subsequent opsonic studies

Methods

Adverse effects (hypotension) induced by the various immunoglobulin preparations were assessed in the cy-

nomolgus monkey. The monkeys were anesthetized by an intramuscular injection of Ketamine hydrochloride (5 mg/kg). Following intubation, anesthesia was maintained by intravenous pentobarbital sodium (5–10 mg/kg as needed). Catheters were inserted in the femoral artery and vein for measurement of mean arterial blood pressure and parenteral administration of the immunoglobulin preparations, respectively.

For the IgM enriched, IgG immunoglobulin concentrates we used an infusion rate of 1 mg/kg/min (IgM) up to a total dose of 50 mg/kg. This rate and dose resulted in severe hypotension within 30 minutes (data to be presented).

Blood pressure measurements were taken from the femoral artery over a 90 minute time period since we have demonstrated that adverse effects, if they result, will occur within this time frame. $C4_a$ anaphylatoxin measurements were performed on plasma from whole blood (anticoagulated with Citrate) obtained at 0, 30, 60 and 90 minutes. The samples were stored at −70° C. C4a measurements were made by radioimmunoassay with kits from Amersham International (Arlington Heights, Ill.).

Definitions

As used herein, the expression antibody (or immunoglobulin) preparation means a collection of therapeutic antibodies comprising at least about 20% by weight of antibodies of the IgM type, the remaining antibodies, if present, being mainly antibodies of the IgG type with trace amounts other types such as IgA, etc. The individual antibodies can be obtained from a variety of sources such as plasma (as described above, for example) or from cell culture systems (e.g. monoclonal antibodies from hybridomas or transformed cell lines). In the examples below, our enriched IgM antibody preparation comprised on average about 30% to 50% by weight antibodies of the IgM type, the remaining antibodies being mainly of the IgG type.

Non-specific complement activation means the activation of the complement cascade by immunoglobulin in the absence of antigen.

Minimal non-specific complement activation means, the generation of less than about 1.0 ug/ml $C4_a$ in an in vitro assay in the absence of antigen. Alternatively, minimal non-specific complement activation means an amount of $C4_a$ generation within about 100% of the amount of $C4_a$ generated using a liquid IGIV at pH 4.25 as a control.

Specific complement activation means the activation of the complement cascade by immunoglobulin (of the IgM or IgG type) in the presence of antigen.

Substantially no loss of specific complement activation, as applied to an IgM enriched antibody preparation, means the antibody preparation is capable of binding to antigen and activating the classical pathway of complement in vitro or in vivo.

Materials

Pd IgM Immunoglobulin Concentrate Preparation

Pd IgM immunoglobulin concentrates were isolated from Cohn fraction III paste (45 kg) suspended in 12.5 volumes of 0.05M acetate buffer pH 3.5–4.0 and mixed at room temperature for 2–3 hours. To the mixture was added 2.0% of caprylic acid by vol/wt at pH 4.8 to remove lipoproteins and prekallikrein activator (PKA) by centrifugation. The extracted caprylate supernatant, after diafiltration and ultrafiltration through PM-30, resulted in low conductivity of 0.03–0.06 mm ho/cm at pH 4.8. Virus inactivation was achieved with 0.3% TNBP/1% Tween-80 at 24° C. for more than 6 hours. The caprylate supernatant was precipitated with buffer system such as tris (0.010) vol. of 1M Tris pH 7.8) or imidazole buffer (0.005 vol. of 1M imidazole pH 7.8) sterile water, adjusting pH to 4.0–4.8 with acetic acid, and further diafiltered/ultrafiltered against water, then adding solid glycine to a final concentration of 0.25M glycine, pH 4.0–4.8. The Pd IgM immunoglobulin concentrates resulted in low PKA (less than 10% of reference) and less than 5% aggregate determined by high pressure liquid chromatography (HPLC). The final IgM-enriched product consisted of 50–60% IgM, 30–40% IgG, 3–5% IgA, on a dry weight basis, in a total 5% protein aqueous solution.

Heat-Aggregated IgG Preparation

A 5% solution of IGIV (Lot #2855-IIB) was used as an appropriate antibody control. A heat-aggregated IgG solution was prepared from the 5% IGIV solution by heating at 62° C. for 1 hour (pH 7.0). Another heat-aggregated IgG solution was prepared from the 5% IGIV solution by heating at 62° C. for 2 hour (pH 4.25).

Heat Treated IgM, IgG Preparation

The heat-treatment of the IgM, IgG preparation in water or glycine (pH 4.0–4.8) ranged from 37° C. to 62° C. for periods of 10 minutes to 8 hours to determine the optimal mode for the treatment.

Assay Methods

Aggregate Determination by High Pressure Liquid Chromatography (HPLC)

Aggregate formation in the native IgM and IgG preparations or induced by heating was determined by high pressure liquid chromatography with TSKG 4000 SWXL gel (7.8×300 mm, 8 μm particle size, Toyo Soda Corporation, Japan) and eluted with 0.05M sodium acetate, 0.20M sodium sulfate, pH 5.0.

Biological and Functional Activity Determination of IgM Immunoglobulin Concentrates

1. Antigenic Determinants of IgM-Enriched, IgG by Radial Immunodiffusion (RID)

The concentration and antigenic determinants of IgM and IgG were determined by radial immunodiffusion (RID) with quiplate system from Helene Laboratories (Beaumont, TX). This method provides an indirect assessment of the integrity of the antibody.

Specific Antigen Binding Sites Study by ELISA against Ps.IT4LPS

The biological activity of IgM immunoglobulin concentrates and mild heat treated IgM concentrates was determined by enzyme-linked immunosorbent assay (ELISA) to quantitate IgM binding to Ps IT4LPS (lipopolysaccharide) and to assess the integrity of specific antigen binding sites. 10 μg of *P. aeruginosa* immunotype 4 LPS in 0.06M sodium carbonate buffer pH 9.5 were coated to Immulon 1 plates (Dynatech Lab) at 37° C. for 3 hours. Each well of the plates was washed twice with PBS—0.05% Tween buffer. The standard Pseudomonas monoclonal antibody and unheat/heat treated IgM concentrates were diluted in 0.01M Tris buffer pH 7.8 containing BSA and added to the plates incubated at room temperature overnight. Each well was washed three times with PBS - Tween buffer. Goat

anti-human IgM alkaline phosphatase conjugate (HyClone, Logan, Utah) was added to the wells, incubated at room temperature for 4 hours and the wells were washed five times with PBS - Tween buffer P-Nitrophenyl phosphate in diethanolamine pH 9.8 substrate solution was added to each well at room temperature for 30 minutes and the A405nm/450nm was read.

2. Specific Complement Activity Determination by Phagocytic Assay

A phagocytic assay was employed to determine the opsonic activity (specific complement activity) of mild heat treated IgM immunoglobulin concentrates. The phagocytic assay employed bacteria (*E. coli* 050:k1) and human phagocytes (PMNs) suspended in tissue culture fluid. The bacteria to PMN ratio was 20 to 1 and 5% (vol/vol) guinea pig serum (GPS) served as complement source. 2.5 ul of IgM concentrates was added to the total assay mixture (500 ul) and incubated at 37° C. for 100 minutes. An aliquot of the assay mixture was added to 9 vol. of distilled water to lyse PMNs and surviving bacteria were enumerated by duplicate agar plate counts.

4. In vitro and in vivo Non-specific Complement Activation assessed by Anaphylatoxin ($C4_a$) Generation

The ability of various immunoglobulin preparations to activate the classical pathway of complement in vitro was assessed by incubation of the respective preparations (1.47 mg IgM or IgG/ml serum) with human serum at 37° C. for 20 minutes and determining the resultant generation of $C4_a$ levels by radioimmunoassay (RIA). The RIA kits were obtained from Amersham (Arlington Heights, Ill.).

Systemic complement activation in vivo was assessed by measuring plasma $C4_a$ levels following parenteral administration of the various immunoglobulin preparations in the monkey. Antibodies raised against human $C4_a$ (RIA kits) partially cross react with monkey $C4_a$, approximately 60%

Results

Table 1 describes the immunoglobulin preparations used in the initial experiments designed to examine the hypothesis that non-specific complement activation may be associated with adverse cardiovascular events following intravenous infusion. To test this hypothesis various immunoglobulin preparations were assayed for their ability to activate the classical pathway of complement in vitro by measuring $C4_a$ generation employing human serum as the complement source. Mean arterial blood pressure in the cynomolgus monkey was measured over a 90 minute period following infusion of the immunoglobulins in order to assess cardiovascular safety. Plasma $C4_a$ levels were also measured in order to ascertain complement activation following infusion

In Vitro Data

A 5% IGIV immunoglobulin (control) solution at 0.2M glycine pH 4.25 did not cause appreciable $C4_a$ generation in vitro when incubated with human serum (<1%, 0.23 ug/ml). The enriched pd IgM, prepared by euglobulin precipitation in a similar glycine buffer pH 4.25, caused substantial $C4_a$ generation in vitro (4.5 ug/ml). To prepare aggregated IgG, a well known activator of complement, we heated the 5% IGIV protein solution at pH 7.0 at 62° C. for 1 hour. This heat treatment resulted in a solution which had 19% pentamer aggregates yet retained over 72% of its antigenic determinants as assessed by RID. This immunoglobulin solution also generated a substantial amount of $C4_a$ (14.0 µg/ml) when incubated with human serum. However, the same IGIV solution when heated at pH 4.25, although generating 58% aggregates (size less than pentamer), did not generate significant amounts of $C4_a$ in vitro (0.56 ug/ml). This IGG solution also lost over 80% of its antigenic determinants as measured by RID.

TABLE 1

Characteristics of Immunoglobulin Preparations

Antibody	Lot #	Buffer	Heat (hrs) 62° C.	RID IgM mg/ml	RID IgG mg/ml	% Aggregates <Pentamer	% Aggregates >Pentamer	$C4_a$ Generated in vitro (Human Serum) (ug/ml)
IGIV (5%)	2855-11-B	0.2M Glycine pH 4.25	0	0	57.0	0	0	0.23
pdIgM	3747-82-E	0.2M Glycine pH 4.25	0	36.0	26.2	0	6.0	4.5
IGIV (HT)	1E053-79-B	0.2M Glycine pH 7.0	1	0	40.9	11.0	19.0	14.0
IGIV (HT)	1E053-66-2	0.2M Glycine pH 4.25	2	0	10.2	58.0	0	0.56

These results demonstrate that both pdIgM, IgG immunoglobulin concentrates and heat-aggregated IGIV at pH 7.0 induced substantial $C4_a$ generation in vitro while native IGIV and heated IGIV at pH 4.25 generated non-significant quantities of $C4_a$ anaphylatoxin.

It was important to determine whether these in vitro measurements of non specific complement activation were associated with adverse cardiovascular effects in the cynomolgus monkey when the immunoglobulin solutions were intravenously infused.

TABLE 2

Acute Effect of Immunoglobulin Preparations on MAP and Plasma $C4_a$ Anaphylatoxin Levels in the Monkey

Antibody	Rate	Dose		Time (min.) 0	30	60	90
IGIV (5%)	10 mg/Kg/min	500 mg/Kg	MAP (mm Hg)	85 ± 5	97 ± 4	96 ± 7	97 ± 6
N = 3			$C4_a$ (ng/ml)	192 ± 91	601 ± 95	385 ± 51	392 ± 180
pdIgM	1 mg/Kg/min	50 mg/Kg	MAP (mm Hg)	97 ± 4	47 ± 3	67 ± 6	64 ± 10
N = 5			$C4_a$ (ng/ml)	253 ± 43	4048 ± 1000	2562 ± 370	611 ± 305
IGIV (5%)	1 mg/Kg/min	20 mg/Kg	MAP (mm Hg)	99 ± 5	47 ± 6	57 ± 7	63 ± 7
pH 7.0 Heated			$C4_a$ (ng/ml)	135 ± 38	4160 ± 262	3100 ± 336	1558 ± 131
N = 3							

TABLE 2-continued

Acute Effect of Immunoglobulin Preparations on MAP and Plasma $C4_a$ Anaphylatoxin Levels in the Monkey

Antibody	Rate	Dose		Time (min.) 0	30	60	90
IGIV (5%) pH 4.25 Heated N = 3	10 mg/Kg/min	500 mg/Kg	MAP (mm Hg)	87 ± 3	85 ± 14	97 ± 7	99 ± 6
			$C4_a$ (ng/ml)	155 ± 17	535 ± 51	372 ± 100	207 ± 30

Table 2 and FIGS. 1–4 presents the in vivo results with these respective immunoglobulin preparations. These results demonstrate that the immunoglobulin preparations which generated substantial $C4_a$ levels in vitro i.e., pdIgM and heat-aggregated IGIV at pH 7.0, caused severe hypotension in the cynomolgus monkey and elevated plasma $C4_a$ levels, while those immunoglobulin preparations which did not generate substantial $C4_a$ in vitro i.e., native IGIV and heat aggregated IGIV at pH 4.25, did not cause hypotension in the cynomolgus monkey and did not greatly increase plasma $C4_a$ levels. Thus, the in vitro assessment of $C4_a$ generation by the various immunoglobulin preparations appeared to be associated with adverse cardiovascular effects in vivo following intravenous infusion.

TABLE 3a

Characteristics of Heat Treated PdIgM IgG Immunoglobulin Concentrate

Antibody	RID IgM	RID IgG	% Aggregates >Pentamer	$C4_a$ Generated in vitro (Human Serum) ug/ml
pd IgM (pH 4.25) (HT 62° C., 2 hrs)	mg/ml 6.58	12.81	47.0	0.27

TABLE 3b

Acute Effect of Heat Treated PdIgM, IgG Immunoglobulin Concentrate on MAP and Plasma $C4_a$ Anaphylatoxin Levels in the Monkey (N = 3)

Antibody	Rate	Dose		Time (min.) 0	30	60	90
PdIgM (pH 4.25) (HT 62° C. 2 hrs)	2 mg/kg/min	100 mg/kg	MAP (mm Hg)	90 ± 3	94 ± 4	98 ± 4	97 ± 5
			$C4_a$ (ng/ml)	155 ± 19	530 ± 25	372 ± 75	251 ± 60

Since an IGIV protein solution heated at pH 4.25 did not cause substantial $C4_a$ generation in vitro and did not cause hypotension when infused intravenously, we reasoned that perhaps heating the pdIgM, IgG immunoglobulin concentrate at pH 4.25 would diminish the non-specific complement activating potential of the IgM, without adversely affecting the IgG which is present in the solution. That is, heating IgG at acidic pH did not result in a solution which activated complement in vitro and did not have adverse effects when infused in the cynomolgus monkey. To test this hypothesis we initially heated the phIgM, IgG immunoglobulin solution at 62° C. for 2 hours and evaluated its $C4_a$ generating potential in vitro. This solution did not generate significant amounts of $C4_a$ in vitro (0.27 ug/ml) and did not cause hypotension or substantial increases in plasma $C4_a$ when infused in the cynomolgus monkey, Table 3a, 3b and FIGS. 1, 2

These results demonstrate that heating (62° C. for 2 hours) an IgM, IgG immunoglobulin concentrate at acid pH (4.25) produces a protein solution which has dramatically diminished non-specific complement activating potential in vitro and does not cause hypotension when infused in the cynomolgus monkey. However, this particular heat treatment (62° C. for 2 hrs) resulted in a loss of more than 80% of the IgM antigenic determinants and a greater than 47% pentameric aggregate formation, Table 3

Thus, although, this heat-treatment diminished the adverse cardiovascular effects associated with intravenous administration, it also appeared to diminish the effector functions of the immunoglobulin. We, therefore, sought to define more closely an optimal heating temperature and incubation time which would result in an IgM, IgG immunoglobulin concentrate which had minimal non-specific complement activating potential while retaining relevant biologic effector functions, i.e., antigen binding, opsonization, etc

During this evaluation, a number of conditions were examined. Table 4 summarizes data concerning the effect of temperature and incubation time on $C4_a$ anaphylatoxin generation in vitro.

TABLE 4

Effects of Temperature and Incubation Time on $C4_a$* Generation in vitro and IgM Antigenic Determinants of IgM IgG Immunoglobulin Concentrates (3747-82-E, pH 4.42)

Incubation Time (Min)	62° C. $C4_a$ µg/ml	62° C. IgM mg/ml	55° C. $C4_a$ µg/ml	55° C. IgM mg/ml	52° C. $C4_a$ µg/ml	52° C. IgM mg/ml	50° C. $C4_a$ µg/ml	50° C. IgM mg/ml	45° C. $C4_a$ µg/ml	45° C. IgM mg/ml	40° C. $C4_a$ µg/ml	40° C. IgM mg/ml
0	10.41	35.82	10.41	35.82	10.41	35.82	10.41	35.82	10.41	35.82	10.41	35.82
10	0.49	17.42	1.08	33.48	2.43	35.16						
20	0.49	14.58	0.51	31.21	1.85	35.14	5.41	35.82				
30	0.02	10.68	0.35	26.77	1.45	35.14						
40			0.60	26.77	1.24	35.14	2.88	35.82				
60	0.07	4.9	0.48	22.50	1.09	35.39	2.12	33.49	5.08	35.68	12.25	35.68
120			0.50	12.54	0.60	35.00	1.49	33.49	4.35	35.68	7.41	35.68
180					0.82	33.41	0.77	32.28	3.25	35.68	5.24	35.68
240							0.67	24.40	2.74	35.68	5.24	35.68
300							0.86	24.40	3.86	35.68	5.60	35.68

TABLE 4-continued

Effects of Temperature and Incubation Time on $C4_d$* Generation in vitro and IgM Antigenic Determinants of IgM. IgG Immunoglobulin Concentrates (3747-82-E, pH 4.42)

	62° C.		55° C.		52° C.		50° C.		45° C.		40° C.	
Incubation Time (Min)	$C4_d$ µg/ml	IgM mg/ml	$C4_d$ µg/ml	IgM mg/ml	$C4_d$ µg/ml	IgM mg/ml	$C4_d$ µg/ml	IgM mg/ml	$C4_d$ µg/ml	IgM mg/ml	$C4_d$ µg/ml	IgM mg/ml
480									4.13	35.68	5.26	35.68

*Control (no exogenous immunoglobulin) $C4_d$ levels have been subtracted from all reported values.

PdIgM, IgG immunoglobulin concentrates (50% IgM, pH 4.42) heated at 62° C. for 10 minutes caused non-significant $C4_d$ generation in vitro (0.49 ug/ml) but approximately 50% of IgM antigenic determinants were lost. Heating the IgM, IgG immunoglobulin concentrate at 55° C. for 30 minutes decreased $C4_d$ generation to 0.35 ug/ms in vitro and the IgM immunoglobulin retained more than 75% of its antigenic determinants. Heating at 52° C. for 120 minutes decreased $C4_d$ generation to 0.60 ug/ms and immunoglobulin retained more than 98% of its antigenic determinants. Heating at 50° C. for 180 minutes decreased $C4_d$ generation to 0.77 ug/ml and the immunoglobulin retained more than 92% of its antigenic determinants. Immunoglobulin heated at 45° – 37° C. retained substantial $C4_d$ generating potential (>4 ug/ml) and did not demonstrate any decrease in IgM antigenic determinants.

We next examined the effects of pH, IgM concentration and incubation times on $C4_d$ generation in vitro, Table 5. Temperature was held constant at 50° C.

TABLE 5

Effects of pH, IgM Concentration and Incubation Time on $C4_d$ Generation* in vitro and IgM Antigenic Determinants of IgM Immunoglobulin Concentrates at 50° C.

Incubation	50% IgM pH 4.42		50% IgM pH 4.24		20% IgM pH 4.25	
Time (Min)	$C4_d$ µg/ml	IgM mg/ml	$C4_d$ µg/ml	IgM mg/ml	$C4_d$ µg/ml	IgM mg/ml
0	7.05	37.90	5.45	37.90	5.00	10.07
15	2.76	37.90	1.22	37.90	1.26	10.07
30	2.52	37.90	0.98	35.56	0.88	10.38
45	2.12	37.90	1.03	35.56	0.96	10.38
60	2.08	36.73	0.54	33.27	0.94	9.77
90	1.74	37.90	0.98	33.27		
120	1.20	35.56	0.79	28.81	0.94	8.32
150	1.03	36.73				
180	0.88	37.90	0.56	26.65	0.90	6.1

*Control (no exogenous immunoglobulin) $C4_d$ levels have been subtracted from all reported values.

Pd IgM immunoglobulin concentrates containing 50% IgM at pH 4.42 heated at 50° C. for 3 hours resulted in a decrease in $C4_d$ generation from 7.06 µg/ml to 0.88 µg/ml and fully retained IgM antigenic determinants. Pd IgM immunoglobulin concentrates containing 50% IgM at pH 4.24 heated at 50° C. for 60 minutes caused $C4_d$ generation in vitro to decrease to 0.54 µg/ml and still retained significant IgM antigenic determinant (88%). IgM concentrates containing 20% IgM at pH 4.25 heated at 50° C. for 30 minutes caused $C4_d$ generation to decrease to 0.88 µg/ml with little loss of IgM antigenic determinants and further incubation for 180 minutes did not cause any additional decrease in $C4_d$ generation in vitro but resulted in a loss of IgM antigenic determinants (40%).

In order to further evaluate the effects of heating on immunoglobulin effector functions we assayed antigen binding activity of IgM to Ps. IT4 lipopolysaccharide under various heating conditions. These results are summarized in Table 6.

TABLE 6

Effects of Temperature and Incubation Time on Antigenic Determinants and Specific Antigen Binding Activity of Pd IgM Concentrates.

	Heat		RID IgM	ELISA α Ps IT4 LPS	Specific Activity α LPS
Sample	°C.	Min.	mg/ml	mg/ml	mg/mg IgM
3747-82-E (pH 4.42)	—	—	36.0	0.542	0.015
1B107-58-1	62	10	17.42	0.400	0.023
1B053-62-6	62	120	6.58	0.040	0.006
1B107-62-3	55	30	26.77	0.518	0.019
1B107-62-5	55	60	24.61	0.364	0.015
1B107-72-9	52	180	35.00	0.483	0.014
1B107-72-11	52	210	32.32	0.455	0.014
1B107-63-7	50	180	32.38	0.427	0.013
1B107-67-15	45	480	35.68	0.604	0.017
1B107-70-1	50	360	32.90	0.419	0.013

Pd IgM, IgG concentrates heated at 62° C. for 120 minutes adversely affected the IgM antigenic determinants, resulting in a loss of more than 90% of specific antigen binding activity, and also a 3 fold reduction in specific activity (α LPS/IgM). Samples treated at the lower temperatures all retained significant specific antigen binding activity and non-significant decrease in specific activity.

We next examined what effect hating had on opsonic activity, another important indicator of biologic effector function. These results are summarized in Table 7.

TABLE 7

Effect of Temperature and Incubation Time on Opsonic Activity of 50% IgM concentrate

Incubation	LOG10 CFU Reduction of *E. Coli* 050:K1 °C.					
Time (min.)	62	55	50	45	40	37
0	3.12	2.86	2.86	2.86	2.86	2.86
10	0.25	2.81				
20	0.19	1.79	3.23			
40		0.55	3.35			
60	0.17	0.42	3.27			
120		0.51	3.07			
180			2.71			
240			2.19			
300			2.18			
480				3.09	3.21	3.09
5% Guinea Pig Serum Only	0.15	0.41	0.41	0.41	0.41	0.41

Unheated IgM significantly enhanced bacterial killing. IgM, IgG concentrates heated at 62° C. for 10 minutes lost substantial opsonic activity. Concentrates heated at 55° C. had diminished activity at 20 minutes and lost substantial opsonic activity at 40 minutes. Heating at 50° C. slightly reduced opsonic activity over time but substantial opsonic activity still remained at 5 hours.

Heating at temperature between 45°-37° C. did not diminish opsonic activity over hours.

Opsonic activity of the IgM, IgG immunoglobulin concentrate heated at 50° C. for 3 hours was also assessed in a phagocyte chemiluminescence assay against *E. coli* 050:K1, FIG. 5. Heating IgM at 50° C. for 3 hours leaves intact the ability of IgM to promote chemiluminescence and phagocytic killing of the bacteria.

Since IgM, IgG immunoglobulin concentrates heated at 50° C. for 3 hours retained effector functions i.e., opsonophagocytic activity, antigenic binding sites, etc., and demonstrated diminished non-specific complement activation in vitro ($C4_a$ generation), we assessed the cardiovascular effects of this preparation following intravenous infusion in the cynomolgus monkey. This data is summarized in Table 8.

TABLE 8

Acute effect of heat treated IgM, IgG immunoglobulin concentrates on MAP and plasma $C4_a$ anaphylatoxin levels in the monkey (N = 3).

	Time (min.)			
	0	30	60	90
MAP (mmHg)	92 ± 7	85 ± 5	88 ± 9	93 ± 7
$C4_a$ (ng/ml)	85 ± 17	326 ± 102	500 ± 52	685 ± 61

IgM, IgG heated at 50°-51° C. for 3 hours.
Rate 1 mg/kg/min
Dose 50 mg/kg

Severe hypotension was not observed in these monkeys following infusion of the immunoglobulin concentrates and plasma $C4_a$ levels were much diminished compared with animals infused with the unheated IgM preparation (Table 2).

Discussion

The parenteral administration of IgM enriched IgG (IgM, IgG immunoglobulin concentrates) is associated with serious side effects including severe systemic hypotension in the cynomolgus monkey. The mechanism whereby IgM, IgG concentrate infusion elicits these adverse effects is not presently known.

In these experiments, however, we have shown that the ability of various immunoglobulin preparations to induce systemic hypotension is related to their capacity to activate the classical complement pathway. That is, immunoglobulin preparations which activate the classical pathway of complement in vitro, (i.e., pdIgM and heat-treated IgG at neutral pH) elicit systemic hypotension when administered intravenously to the monkey. While immunoglobulin preparations which do not activate the classical pathway of complement in vitro, (e.g., heat-treated pdIgM, native IgG and heat-treated IgG at acid pH) do not elicit any adverse hemodynamic effects when administered intravenously to the monkey.

It therefore appears that the in vitro assessment of complement activation (classical pathway) of various immunoglobulin preparations has predictive value for estimating the capability of these preparations to elicit adverse effects in the monkey. Whether this is a direct cause and effect relationship or these phenomenon are merely temporarily related has not been determined. Furthermore, and of greater importance, we have shown that mild heat-treatment of pdIgM, IgG immunoglobulin concentrates diminishes its potential to non-specifically activate complement in vitro and this terminal process treatment greatly decreases it's ability to induce adverse the cynomolgus monkey.

Since antigenic determinants and specific antigen binding sites are retained with less harsh heat-treatment (at the presently preferred temperature of about 50° C. for 3 hours) it would appear that antibody integrity has not been compromised at these temperatures while non-specific complement activating potential has been dramatically diminished, thus this treatment would result in a much better product.

It has now been demonstrated that IgM-enriched, IgG immunoglobulin concentrates can be heat treated at elevated temperatures for extended periods of time without significant loss of antigenic determinants or specific antigen binding sites. The preparations still retain opsonophagocytic activity while exhibiting dramatically diminished non-specific complement activity. Consequently, through suitable heating temperatures for suitable periods of time at suitable pH, suitable protein concentration and suitable stabilizer, the non-specific complement activity can be diminished in the IgM-enriched, IgG immunoglobulin concentrate product while retaining the antigenic determinants, specific antigen binding sites, specific complement activity when bound to antigen (opsonophagocytic activity) and therapeutic integrity of pdIgM, IgG immunoglobulin concentrates product.

Given the above disclosure, it is thought that variations will occur to those skilled in the art. Accordingly, it is intended that the above disclosure should be construed as illustrative and the scope of the invention should be limited only by the following claims.

We claim:

1. A method of treating an antibody preparation comprising antibodies of the IgM type, the method comprising the step of subjecting the preparation to a gentle heating step at a temperature ranging from 45° C. to 55° C. in an aqueous solution having a pH of 4.0 to 5.0 for at least 10 minutes to minimize any non-specific complement activation without substantial reduction of the specific complement activation activity of the IgM.

2. The method of claim 1 wherein the preparation is heated for about 1 to 3 hours at a temperature of about 50° C.

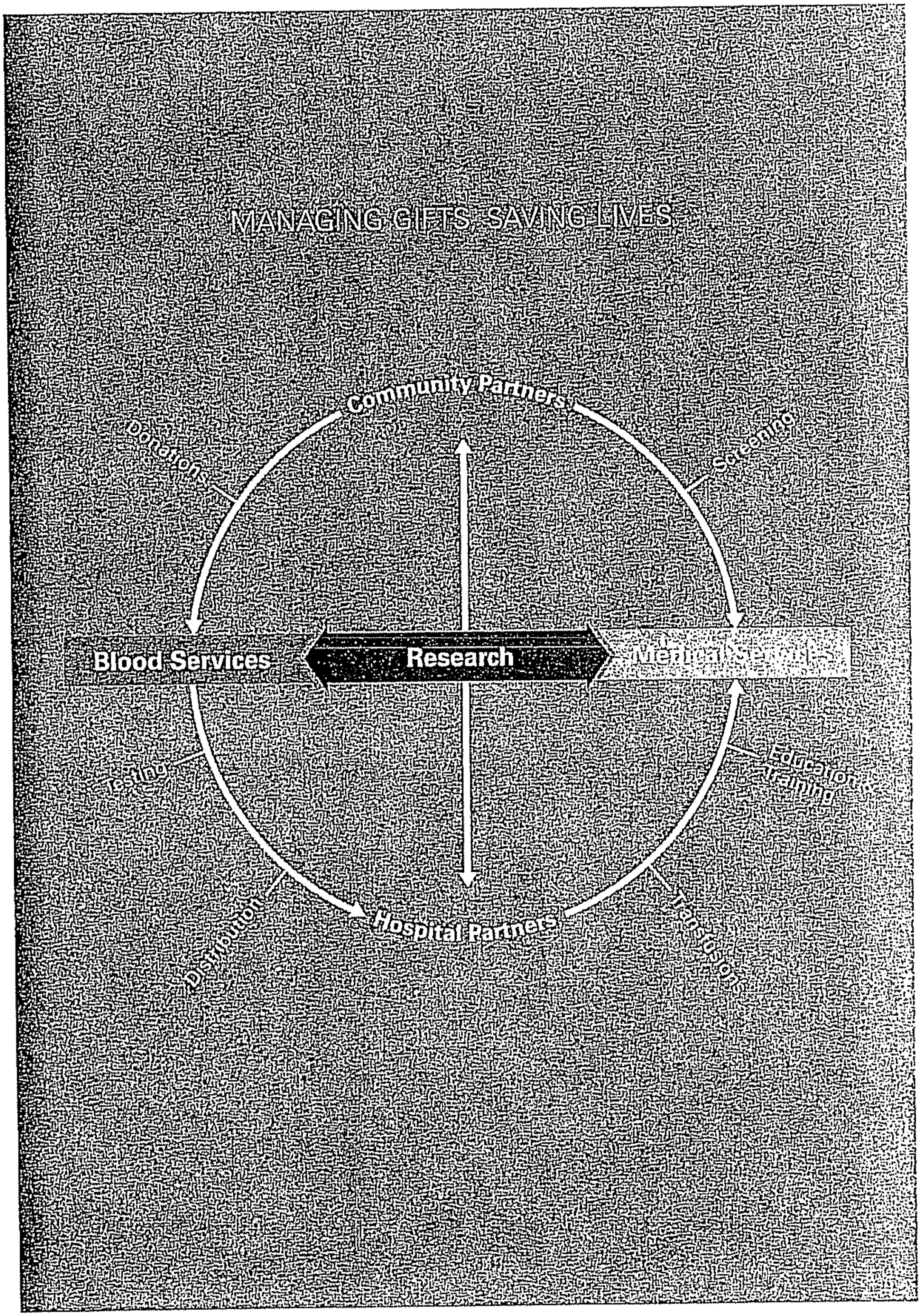
3. The method of claim 2 wherein the pH is about 4.24 to 4.42.

* * * * *

EXHIBIT 5

New York Blood Center

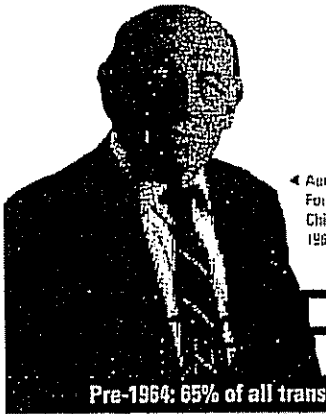
One decade at a time


MANAGING GIFTS, SAVING LIVES
Community Partners
Donation
Screening
Blood Services
Research
Medical Services
Testing
Education
Training
Distribution
Hospital Partners
Transfusion

XL

40 YEARS

FOUR DECADES During NYBC's first decade, man first set foot on the moon. During the second decade, the VCR was invented and the world was captivated by Star Wars. During the third, the fall of the Berlin Wall marked the end of Communism in Europe. And during the fourth decade, the United States both enjoyed unprecedented economic prosperity and mourned the tragedy of terrorist attacks. Many events changed the face of the planet during the past four decades, including significant contributions by NYBC locally, nationally and globally. The contributions to community health during this vibrant period paved the way for the significant impact NYBC will continue to make moving forward With NYBC refining its organization into Blood Services, Research and Medical Services functions to better focus on improving community health, the future holds even greater promise.



◄ Aaron Kellner MD
Founder of New York Blood Center
Chief Executive Officer & President
1963-1989

►
(left to right)
- New York Blood Services (Center West)
- Inter-County Blood Services (now Long Island Blood Services)
- Hudson Valley Blood Services
- New York Blood Center (Center East)

First Decade (1964-

Pre-1964: 65% of all transfused blood purchased from skid-row derelicts

1964 – Support generated by the Community Blood Council of Greater New York and the Public Health Committee of the New York Academy of Medicine's year-long study of Human Blood in New York City leads to NYBC collecting blood with Aaron Kellner, MD as our President. As we continued, our Greater New York Blood Program started to supply area hospitals.

Research underway included: Dr. Kellner studying the relation of fat in the blood to heart disease and Dr. Fred Allen studying blood groups and human genetics.

1965 – First centrally administered Blood Donor Program established for municipal employees (now 22,000 annual donations).

First rare blood registry established for NY metropolitan area (previously this blood was obtained from Seattle, Milwaukee and Miami).

1966 – Four National Institutes of Health (NIH) grants for a Research Resource Program: Blood Group Antigen-Antibody Reactions, Mobilization of Lipids in Living Animals, and Relation of Ferritin to Iron Metabolism.

1967 – AMA's *Today's Health* reports on NYBC's progress in cryobiology in "Super Cold: The Hottest Thing in Science."

1968 – Education and training for medical professionals begins with an emphasis on blood group serology and blood bank technology as well as medical graduate student programs.

The Laboratory of Virology under the direction of Alfred Prince, MD establishes serum hepatitis lab testing.

1968 – What comes to be known as the Euroblood Collaboration with Red Cross centers in Switzerland, West Germany and Belgium begins, accounting for 10-37% of NYBC's supply.

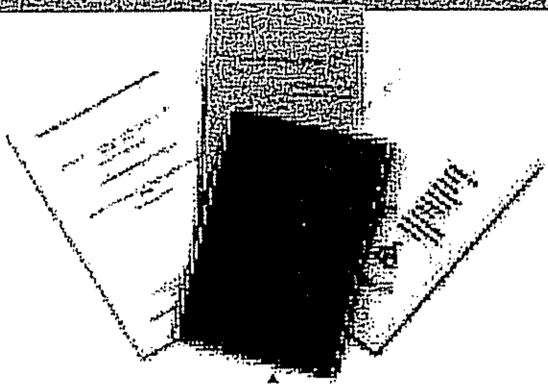
1969 – Greater New York Blood Program combines the services and facilities with Community Blood Council and the American Red Cross.

196,008 units of blood collected from over 172,000 donors.

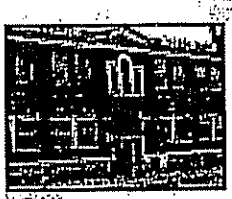
77% of blood is collected from mobile blood drives at businesses, schools, religious groups, community organizations and government offices.

NYBC becomes the first USA blood center licensed to fractionate transfusable products from plasma.

Dr. Arthur Rowe, ► one of the nation's foremost authorities on cryobiology, operates a device which instantly froze blood droplets to the consistency of sand.



▲
Landmark study from New York Academy of Medicine that recommended the creation of NYBC as a community resource. Also pictured are early reference materials.



Dr. Fred H. Allan Jr., Director of Laboratories
Head of NYBC's teaching staff

1973)

1971 – Automation begins to pave the way for reduced processing times and productivity improvements.

1972 – Marked the first five years of the collaborative Greater New York Blood Program with cumulative collections of 315,453 units (collections up 21%) ... 423,844 transfusable units distributed to area hospitals.

– Strong support from 410 volunteers across region recruited through American Red Cross partnership.

– Donor Groups 4,500 with as few as 3 members and up to 35,000 employees.

– NYBC plans a donor room at the World Trade Center office complex. This site was well utilized by the Wall Street community through 1979, then opened in a larger space with more capacity before closing in 1998.

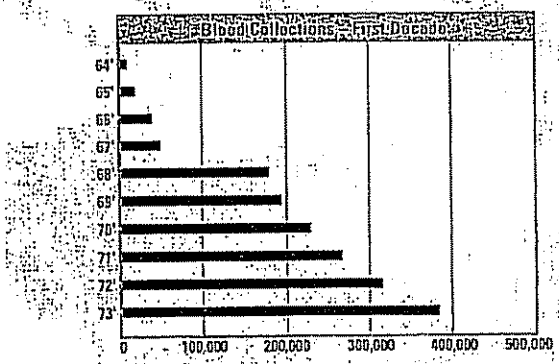
– Better understanding of liver metabolism and malfunction of protein production in the liver comes out of Dr. Colvin Redman and colleagues work in the Membranes Laboratory.

1973 – Renovation of 310 East 67th Street headquarters completed through support of the Capital Fund Campaign Committee chaired by then President of NY Telephone, William Ellinghaus.

– 837 units are donated to the NY-NJ Regional Transplant Program (RTP) supported primarily by centralized resources from NYBC.

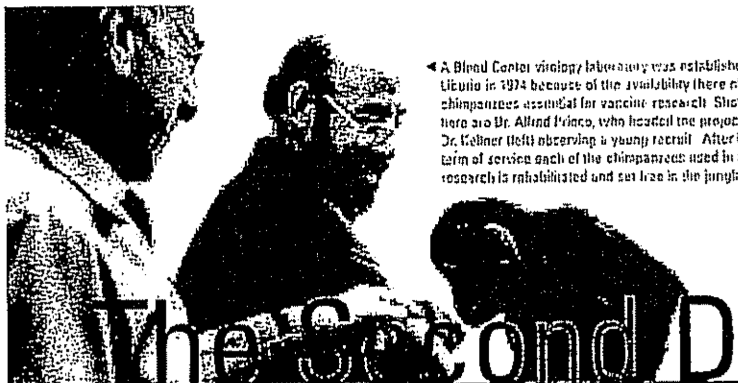
– NYBC/Community Blood Council organizes 1-Year Post Doctoral Clinical and Research Training Program for Blood Bank Directors in recognition of an acute shortage of qualified professionals.

– The first "Groupamatic 360" automated blood testing machine is used by NYBC to perform 12 tests on 360 blood samples per hour – greatly reducing human error and increasing productivity.



One of NYBC's first self contained mobile units to collect blood, was able to process more than 10 donors an hour.

(background picture)
Reconstruction of existing building to house NYBC's multifaceted operation cost more than $4 million over a two-year period.



◄ A Blood Center virology laboratory was established in Liberia in 1974 because of the availability there of chimpanzees essential for vaccine research. Shown here are Dr. Alfred Prince, who headed the project, with Dr. Kellner (left) observing a young recruit. After its term of service each of the chimpanzees used in the research is rehabilitated and set free in the jungle.

▲ Lindsley Fiske Kimball, AB, PhD, LLD
NYBC Trustee

The Second Decade (19

1974 — NYBC organized into five areas: Blood/Blood Derivatives, Research, Transplantation, and Education (and central administrative services).

— NYBC adds a second "Groupamatic 360" to further expand testing and improve safety.

— 70 Research Grants bring in $3.25 million dollars for projects.

— Murray Sargent Auditorium dedicated at 310 East 67th Street in honor of the Blood Transfusion Association (BTA) President.

— NYBC's First Vice President, Lindsley Fiske Kimball, is honored as the Research Institute is named for his efforts.

1975 — Research increases staff significantly to 140 including 45 doctoral level scientists.

— NYBC Education Department develops first Blood Bank Directors Training Program for physicians along with Technician Training Program for licensed technicians and Summer Research Fellowship Program for college students.

— NYBC organizes the first L.C. Dunn Lecture Series in honor of the late Columbia University Professor who was one of the world's leading geneticists and a charter member of NYBC's Community Blood Council Scientific Council.

1976 — Euroblood units contribute 199,328 units to the total 819,890 units collected as nearly 300 hospitals served by NYBC.

— BLOODNET, one of the first computer programs for testing, labeling, storing and shipping blood, is established as a joint venture between the blood program and the Operations Research Laboratory (Eric Brodheim, working with Dr. Fred Allen).

— 90 research papers are published, including important studies by Pablo Rubinstein, MD of the Laboratory of Immunogenetics and Wolf Szmuness, MD of the Laboratory of Epidemiology.

1977 — NYBC officially changes its name from Community Blood Council of Greater New York to New York Blood Center.

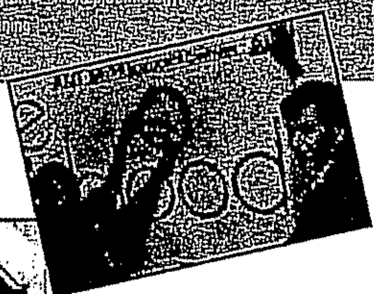
— NYBC's single donor cytopheresis program (for separating platelets and white cells while returning plasma and red cells to the donor) expands to over 9000 volunteer donors typed for HLA antigens on their white blood cells and platelets.

— NYBC's Blood Derivatives Program process a record 53,741 liters of plasma for clinical use in area hospitals.

1978 — The first low cost HBV (Hepatitis B) vaccine licensed, enabling a technology transfer and training by Dr. Alfred Prince and the staff of the Laboratory of Virology. Over 75 million doses produced for public sector immunization at a cost of 50 cents prevent an estimated 1 million cases of liver cancer.

1979 — NYBC completes financing of the expansion of its Melville Laboratories with a $12.5 million dollar privately placed bond issue with the Dormitory Authority of New York State.

William I. Spencer, Chairman of the Board, gave his time, ideas, leadership and blood to NYBC. Mr. Spencer is the former president of Citicorp.
▼

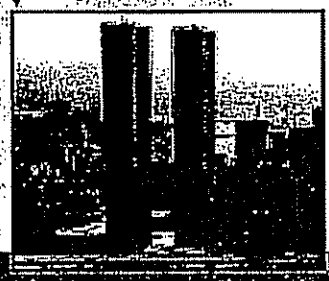


▲ "You cannot get AIDS by giving blood" was the message added to AT&T campaign slogan. It was added to dispel unfounded fear potential donors may have of contracting AIDS, says John Driscoll, division manager, personnel, and blood drive coordinator (right). Mr. Driscoll and his assistant coordinator Marie Roan helped spearhead AT&T's efforts which have resulted in steady donation increases, during the summer of 1983, a remarkable 113 percent of goal.

A home care convenience kit containing a coagulation five-pack and equipment for self-administration by hemophilia patients being delivered by a Long Island Blood Services driver. Upon receipt of a physician's prescription, LIBS makes regular deliveries of these kits to offices and homes of hemophilia patients.
▼

(facility pictured)
NYBC's Blood Derivative Program; shown is one of the fractionation units that were part of the plasma processing operation which included centrifuges, pumps and refrigeration in a single uninterrupted flow

NYBC operated a donor room at the World Trade Center Office Complex (4 and 5 World Trade Center) which closed in 1993



74-1983)

– NYBC signs a long term agreement for the preparation of blood plasma derivatives with the American Red Cross.

– NYBC pioneers use of bar-coding in blood banking operations, now standard throughout the world in reducing possibility of human error and speeding the blood product processing.

1980 – Facility for production of plasma derivatives opens as part of NYBC's Long Island Blood Services operation, and annual production capacity increases from 60,000 liters to 300,000 liters along with preparation of new plasma products with therapeutic potential.

– NYBC's Derivatives Program receives a $976,000 grant by the National Heart, Lung and Blood Institute for a three year study of investigative drug interferon.

– The Laboratory of Virology, under the direction of Dr. Prince, acquires a modern breeding facility for chimpanzees in Liberia, Africa to protect the endangered species and provides experimental animals for hepatitis studies.

1981 – Blood usage increasing at a rate of approximately 5% per year largely due to advances in medicine and surgery and the aging of the population.

– NYBC's first volunteer blood donor campaign (headed by NYBC's Chairman of the Board, at the time also President of Citibank, the late William Spencer) achieves a first year increase of 7% in collections and produces a record 250,000 platelets primarily for treating cancer patients.

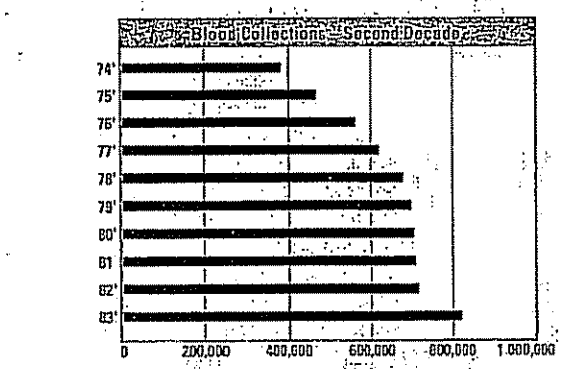
– NYBC's Blood Program is first to create the Cell Isolation and Analysis Laboratory with a fluorescence activated cell sorter to separate, classify and study blood lymphocytes.

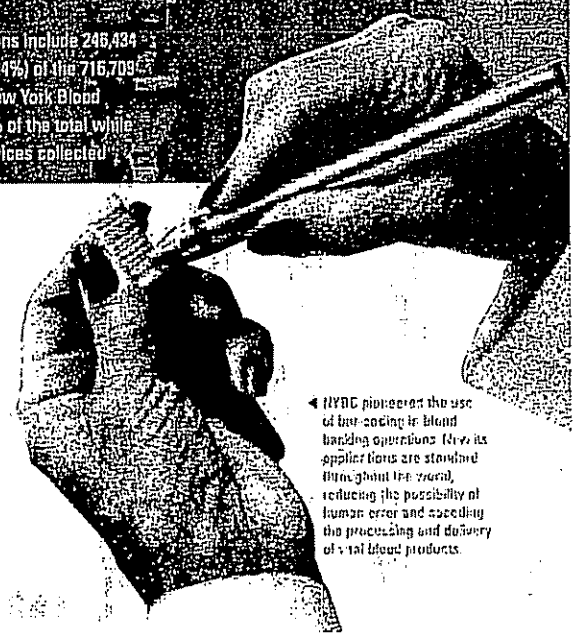
1982 – Blood Collections include 246,434 units from Eurobiood (34%) of the 716,709 unit total collected. New York Blood Services collected 30% of the total while Long Island Blood Services collected 17%; New Jersey Blood Services collected 10% and Hudson Valley Blood Services collected 9% of the total.

– Dr. Cladd Stevens begins AIDS research project while Dr. Pablo Rubinstein studies changes in the immune system of AIDS patients with Karposi's sarcoma.

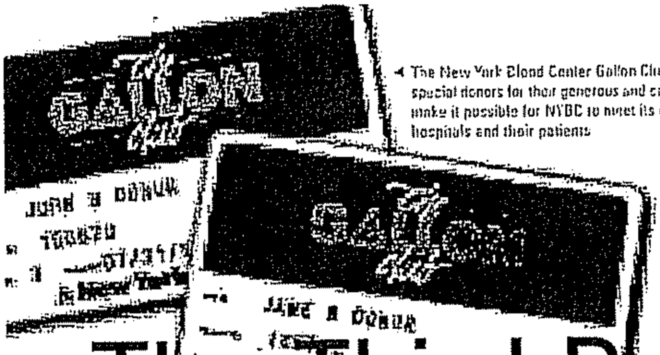
– Drs. Prince, Neurath and Horowitz begin studies on the inactivation of viruses.

1983 – NYBC pioneers a Home Care Convenience Kit for hemophilia patients containing coagulation materials and self-administration equipment. Today in cooperation with the Hemophilia Consortium, NYBC's Hemophilia Services division has provided products and services to over 50,000 patients – locally and regionally.





◀ NYBC pioneered the use of bar-coding in blood banking operations. Now its applications are standard throughout the world, reducing the possibility of human error and speeding the processing and delivery of vital blood products.



◄ The New York Blood Center Gallon Club was developed to recognize special donors for their generous and caring spirit. Gallon Club members make it possible for NYBC to meet its commitment to our region's hospitals and their patients.

The Third Decade (1984

1984 — Total Blood Collections reach 772,359 units with Euroblood supplying 289,735 units (the largest number of Euroblood units provided due to the AIDS crisis).

— NYBC's Transfusion Medicine Training program continues to train physicians, now responsible for training 30% of transfusion medicine professionals in our region's hospitals. High school and undergrad college students also receive training from a grant by Exxon Corporation and the Department of Health and Human Services.

1985 — Public health officials estimate 1.5 million Americans are infected with AIDS virus with an estimated [illegible]% of AIDS cases due to transfusion-related incidents.

— NYBC introduces confidential self-exclusion for all blood donors, pioneered by Dr. Johanna Pindyck. Procedure becomes a global model that impacts blood safety during a critical time of AIDS-related concerns and fears.

— Eight major AIDS research projects by NYBC are funded with grants totaling $2 million dollars.

— Solvent Detergent Viral Inactivation Process to inactivate the viruses of hepatitis B, hepatitis non-A, hepatitis non-B and HIV in plasma is patented and licensed by the FDA for use as a biological product manufactured at Melville Biologics, the Blood Derivatives Program of NYBC.

1986 — Revenues pass the $100 million dollar mark with 95% coming from providing blood products and services to hospitals.

— Total blood collections at 1,810,670 with a record distribution of 1,258,253 units of blood and components.

1987 — NIH and Government grants total $5.4 million dollars for research projects with Foundation and Corporate grants of $1.6 million dollars.

1988 — NYBC focuses on strengthening services under the leadership of John S. Reed of Citibank as Chairman of the Board and Norman O. Selby as Chief Operating Officer.

1989 — John Adamson, MD succeeds Aaron Kellner, MD as New York Blood Center's 2nd President and CEO.

1990 — NYBC develops SAFEBLOOD, the first laboratory management system to improve blood safety.

— FDA approves license for NYBC's patented process to inactivate viruses in Factor VIII products for hemophilia patients (SD) solvent detergent treated factor VIII.

▲ Plasma control panel which is used to monitor and control plasma and plasma fractions.

Robert Neurath, PhD, one of many scientists at NYBC dedicated to the eradication of blood-transmitted disease ►

(background picture)
This picture never changes: over 2000 volunteers such as this donor are needed every day to keep our hospitals supplied with blood and blood components

◀ Automated blood typing on the Groupamatic machine reduces the possibility of human error

-1993)

— Transfusion Medicine course developed for pathology and hematology residents.

— Gallon Club Donor Recognition Program developed to recognize frequent donors.

1991 — NYBC's Clinical Services group triples the number of PAT (Perioperative Autologous Transfusion) procedures from 250 to 830, reducing usage of donated blood in surgery by capturing and returning the patient's blood. Over 5000 procedures now performed annually.

— NYBC develops patented technology and conducts clinical trials for universal Type O blood converted from Type B blood.

— NYBC processes over 400,000 liters of plasma processed for the production of albumin, gamma globulin and Factor VIII.

— NYBC's Education Department develops first foreign certification in transfusion medicine.

1992 — Laboratory of Epidemiology launches a 3 year study (spearheaded by Dr. Beryl Koblin) relative to the efficiency of HIV-1 vaccine trials.

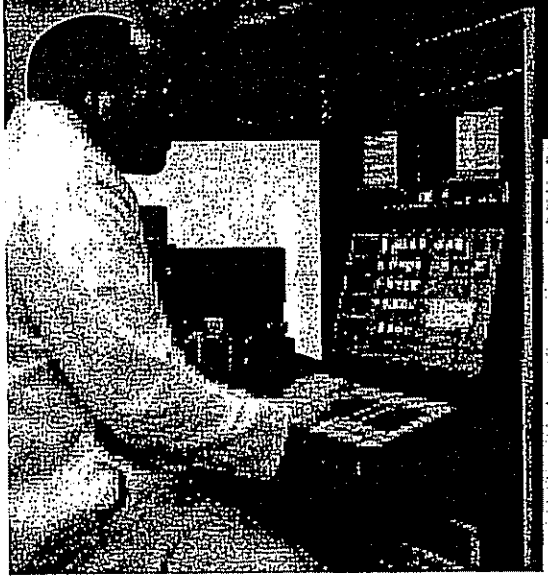
— Research progress by Dr. Pablo Rubinstein continues in the therapeutic use of stem cells harvested from umbilical cord blood in work supported by a grant from NIH.

1993 — Total blood collections of 725,534 pints are supported by 214,360 Eurobloood units.

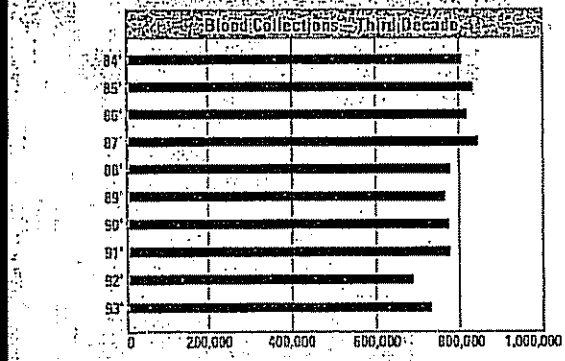
— NYBC continues identification of blood group antigens and clones gene that determines the Duffy blood group and Xg antigen. New insights into autoimmune disease and malaria prevention emerge.

A special biocontainment facility at NYBC provides safe environment for basic research on highly infectious viruses.

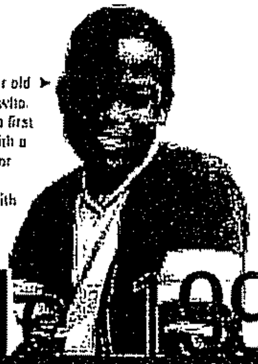
— Therapeutic Apheresis procedures (exchanging plasma and blood components in patients with blood disorders increased by 13% to 915 procedures.



◀ Dr. Joy Valinsky, assistant director, Cell Isolation-Flow Cytometry, operating the fluorescent activated cell sorter, which is a laser based device used for a rapid automated analysis of blood cells. It is useful in assessing the status of immune systems such as those of AIDS victims. When white blood cells are tagged in the laboratory with specific antibodies, this device identifies, classifies and characterizes different cell populations.



◄ Dedication of a new Blood Mobile. Working together with volunteer leaders to inspire "Giving"



Keone Penn is a 14 year old African-American boy who, four years ago, was the first person to be treated with a cord blood transplant for sickle cell disease – a treatment pioneered with the help of NYBC ►

The Fourth Decade 199

1994 – Laboratory of Stem Cell Biology established to investigate stem cell function at cellular and molecular levels.

Researchers in the Laboratory of Virology and Parasitology achieve a breakthrough in hepatitis C research, visualizing the naked virus.

1995 – Restructuring of Melville Biologics, formerly a division of NYBC, into a separate for-profit subsidiary which becomes publicly traded company in 1997 called V.I. Technologies (VITEX).

NYBC's Placental Cord Blood Program (now known as the National Cord Blood Program) surpasses the 6,000 unit milestone of cord blood units collected & stored.

1996 – Revenues surpass the $200 million dollar level with close to $50 million coming from Testing and Specialty Services and $25 million from royalties and contributions.

NYBC files with FDA for clinical trials for the Placental Cord Blood Program, which has shown success in over 200 unrelated gravely ill patients.

1997 – Blood Services holds over 6,000 blood drives to meet the blood needs of our region.

NYBC establishes independent Quality Assurance function and outsources viral and ABO/Rh testing operations.

Hematopoietic Stem Cell Laboratory processes over 900 units of stem cells.

1998 – Robert L. Jones, MD becomes New York Blood Center's 3rd President and outlines a comprehensive strategic plan to examine, re-shape and implement a decentralized blood services organization to improve our effectiveness as a community blood service.

Laboratory of Parasitology, under Dr. Sara Lustigman, makes important progress at identifying an agent to prevent one of the most common malaria-causing parasites from binding to red cells.

Dr. Pablo Rubinstein and colleagues publish a landmark paper in the New England Journal of Medicine documenting the world's largest clinical experience of outcomes among patients using placental cord blood for stem cells as compared to bone marrow.

1999 – Total Blood Collected is 633,044 units with 200,877 units coming from Euroblood.

NYBC scientists have identified over 1/3 of the currently known (over 450) blood group antigens.

2000 – Local blood donations increase 10% over the previous year.

NYBC expands transfusion services provided to Westchester Medical Center to include NYBC staff and management at Nyack Hospital.

Yankee High School Blood Donor Championship brings in more than 50,000 blood donations increasing the cumulative contribution level to over 200,000 since 1996.

Fibrinogen research is a leading focus of research at NYBC's Lindsley F. Kimball Research Institute and helped heart disease research.

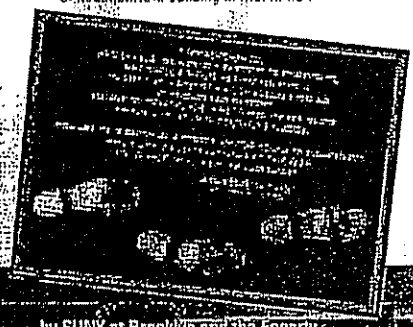
◄ Contributing to the design and evolution of prevention strategies against HIV infection including vaccines

▲ NYBC's Distribution/Hospital Services area in Westbury was designed to process all of our region's blood needs if necessary

Ensuring blood safety through advances in bacterial detection ►

(background picture)
NYBC's National Cord Blood Program Processing and Storage Laboratory; shown is the BioArchive® System used for dry preservation of cord blood units which uses microprocessor controls to manage over 3,000 cord blood units which are ready to be sent anywhere in the world for patients in need

▼ NYBC dedicates memorial to victims of World Trade Center terrorist attack located at the main entrance of headquarters building in Manhattan



4-2003)

2001 – The terrorist attack on the World Trade Center brings out thousands of donors and volunteers at this time of great sadness for our country.

– Brooklyn/Staten Island Blood Services increases donation by 28% over the previous year. This new blood services division of NYBC started in 1999.

– Long Island Blood Services division program aimed at prospective jurors yields 3,000 donations in its first year.

– New Jersey Blood Services division brings platelet collections to mobile units.

– Keone Penn, 14-year-old cured of sickle cell disease by a cord blood unit from the National Cord Blood Program in 1999, honored at NYBC's BiAnnual Chairman's dinner.

– NYBC collaborates with the Academic Medicine Development Corporation (AMDeC) in the New York Cancer Project, a 20 year study to learn how various factors affect a person's chance of developing cancer.

2002 – Free genetic screening for hereditary hemochromatosis (excess iron) piloted in our Long Island Blood Services division.

– Special Donor Services passes the 175,000 mark with people enrolled in the National Donor Marrow Program.

– Complement Biology Research Program receives a grant from NIH and the American Heart Association to study the immune response that seeks to destroy mismatched transfused blood.

– NYBC's Euroblood program ends due to concern of Mad Cow Disease.

– NYBC achieves milestone settlement dispute with Solvent Detergent (SD) licensees involving 19 separate actions in 3 European countries.

2003 – Total Collections of 644,239 units with 115,953 from other US blood sources and 39,769 from Euroblood.

– The National Marrow Donor Program at NYBC celebrates the 500th stem cell donation.

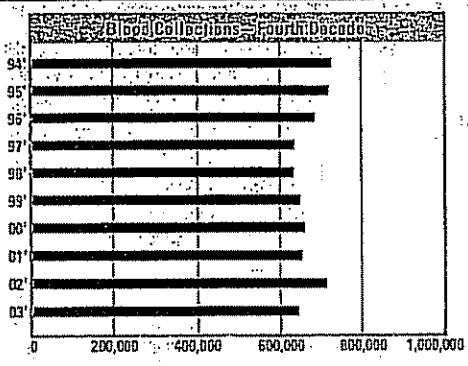
– NYBC's Translusion Medicine program continues with an international training program for transfusion safety sponsored by SUNY at Brooklyn and the Fogarty Foundation of NIH.

– The Laboratory of Biochemical Virology continues efforts to develop a topical microbicide for the prevention of sexually transmitted diseases as Phase I human clinical trials get underway.

– Dr. Shibo Jiang of the Laboratory of Viral Immunology licenses patent to Trimeris, Inc as an anti-HIV drug targeting HIV surface proteins.

2004 – NYBC's National Cord Blood Program receives the 25,000th umbilical cord blood donation as it continues building inventory to meet the diverse transplant needs.

– NYBC achieves self sufficiency in platelet donations and launches new Donor Relationship Management (DRM) system to better address the needs of blood and platelet donors.



Processing cord blood ▶ donations at the National Cord Blood Program by removing unneeded red cells and plasma

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www.nybloodcenter.org